

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph that begins on page 1, line 30, as follows:

The immunome is composed largely of antigens defined by T-cell epitope cloning (van der Bruggen P, et al. 1991. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 254:1643-47; Gaugler, B., et al. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. *J. Exp. Med.* 1994; 179: 921-30; Kawakami, et al. Cloning of the gene for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc. Natl. Acad. Sci. USA.* 1994; 91: 3515-19; Boel, P., et al. BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. *Immunity* 1995; 2: 167-75. (PMID: 7895173); Van den Eynde, B., et al. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J. Exp. Med.* 1995; 182: 689-98. (PMID: 7544395)), MHC peptide elution (Skipper JC, et al. An HLA-A2-restricted tyrosinase antigen on melanoma cells results from posttranslational modification and suggests a novel pathway for processing of membrane proteins. *J Exp Med* 1996 Feb 1;183(2):527-34; Cox AL, et al. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. *Science* 1994 Apr 29;264(5159):716-9; Pascolo S, et al. A MAGE-A1 HLA-A A*0201 epitope identified by mass spectrometry. *Cancer Res* 2001 May 15;61(10):4072-7), and serological expression cloning (SEREX, Chen, Y. -T., et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc. Natl. Acad. Sci. USA.* 1997; 94: 1914-18; Jager D, et al. Identification of a tissue-specific putative transcription factor in breast tissue by serological screening of a breast cancer library. *Cancer Res* 2001 Mar 1;61(5):2055-61; Sahin, U., et al. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc. Natl. Acad. Sci. USA* 1995; 92: 11810-13; Scanlan, M. J., et al. Characterization of human colon cancer antigens recognized by autologous antibodies. *Int. J. Cancer* 1998; 76: 652-8; Scanlan, M. J., et al. Antigens recognized by autologous antibody in patients with renal-cell carcinoma. *Int. J. Cancer* 1999; 83: 456-64; Scanlan MJ, et al. Humoral immunity to human breast cancer: antigen definition and quantitative analysis of mRNA expression. *Cancer Immunity* 1:4 [epub]), and is catalogued in three databases: the peptide database of T-cell defined tumor antigens

(authored by members of the Ludwig Institute for Cancer Research (LICR) that is available on the website of Cancer Immunity, Journal of the Academy of Cancer Immunology, <http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes>); the SYFPEITHI database of MHC ligands and peptide motifs (available on the website of Biomedical Informatics-Heidelberg, <http://www.bmi-heidelberg.com/syfpeithi/>) and the cancer immunome database available on the website of the LICR (www2.licr.org/CancerImmunomeDB, formerly www.licr.org/SEREX.html).

Please amend the paragraph that begins on page 72, line 1, as follows:

Antigens did not react with sera from normal blood donors (0/33).¹ Abbreviations: BC, breast cancer; CC, colon cancer; ES, Ewing sarcoma; FS, fibrosarcoma; GC, gastric cancer; GL, glioma; HC, hepatocellular carcinoma; LC, lung cancer; LS, leiomyosarcoma; MEL, melanoma; MFH, malignant fibrous histocytoma; OC, ovarian cancer; OS, osteosarcoma; PRC, prostate cancer; RC, renal cancer; RS, rhabdomyosarcoma; TALL, T-cell acute lymphocytic leukemia.² SEREX database ID numbers from the LICR's SEREX database (<http://www.licr.org/SEREX.html>).

Please amend the paragraph that begins on page 74, line 1, as follows:

¹ The LICR's SEREX database ID numbers from <http://www.licr.org/SEREX.html>.

² Abbreviations: BC, breast cancer; CC, colon cancer; HD, Hodgkins disease; GC, gastric cancer; OC, ovarian cancer; PC, pancreatic cancer; RC, renal cancer.

Please amend the paragraph that begins on page 74, line 7, as follows:

¹ Antigens reacted with sera from single sarcoma patient (1/39), but not with sera from normal individuals (0/33). The antigens listed had no matches with existing entries in the SEREX database (<http://www.licr.org/SEREX.html>).

Please amend the paragraph that begins on page 75, line 1, as follows:

The nucleotide sequences of all uncharacterized gene products (NY-SAR-3, -10, -16, -22, -23, -24, -27, -28, -29, -35, -41, -48, -62, -71) have been deposited in the GenBank database (SEQ ID NOs: 1-14, respectively). The cDNA sequences encoding the 72 sarcoma antigens were also compared to sequences deposited in the SEREX database accessible through a website of the Ludwig Institute for Cancer Research (<http://www.licr.org/SEREX.html>). Examination of this database revealed that 21 of the 72 sarcoma antigens defined in this study (29%) were also identified through SEREX analysis of other tumor types (Tables 4 and 5).

Please amend the paragraph that begins on page 78, line 2, as follows:

An analysis of the human genome database, mapped the NY-SAR-35 cDNA sequence to Xq28, approximately 5.9Mbp downstream (3') of the CT10/MAGE-E1 gene and 6.8 Mbp upstream (5') of the NY-ESO-1 gene. The NY-SAR-35 gene is approximately 44 kb in length and spans 6 exons. Analyses of the human genome databases (NCBI GenBank, <http://www.ncbi.nlm.nih.gov/genome>, and Celera Genomics, Rockville, MD, www.celera.com) revealed no genomic sequences of high similarity, suggesting that it is a single copy gene with no additional family members. These results were verified by probing Southern blots of human genomic DNA with the NY-SAR-35 cDNA.

Please amend the paragraph that begins on page 79, line 29, as follows:

To identify a murine orthologue of NY-SAR-35, the putative human NY-SAR-35 protein sequence was used to search a translated nonredundant nucleotide database by using the TBLASTN tool of the NCBI (www.ncbi.nlm.nih.gov/blast/Blast.cgi). A hypothetical mouse protein, termed XP_150408, generated from a conceptual translation of the mouse X chromosome, was found to have 57% identity (49/85 amino acids) with NY-SAR-35. Using nucleotide primers corresponding to sequences encoding XP_150408, 5' and 3' RACE reactions were undertaken by using mouse testis cDNA. By combining 5' and 3' RACE products, a 1,202 bp cDNA was identified (GenBank accession no. AY214130, SEQ ID NO: 133). This cDNA encoded a putative full length mouse protein of 238 amino acids (SEQ ID NO: 134) which is

41% identical to human NY-SAR-35, with conservation of the trefoil and transmembrane domains. This murine NY-SAR-35 (mNY-SAR-35) cDNA sequence was used to search mouse genome sequences (www.ncbi.nlm.nih.gov/genome/seq/MmBlast.html) yielding an identical genome sequence, NW 042622, from mouse chromosome X. Analysis of this sequence showed the mNY-SAR-35 gene is composed of approximately 42,600 nucleotides and seven exons.

Please amend the paragraph that begins on page 80, line 16, as follows:

There are four ATG codons in exon 1 of the NY-SAR-35 gene. It is expected that the fourth ATG codon in the full length sequence of NY-SAR-35 is the first ATG codon of the translated NY-SAR-35 sequence. It appears then that the predicted protein has two interesting domains. The protein revealed two distinctive hydrophobic domains followed by two hydrophilic turns. One hydrophobic domain is a signal peptide, which are predicted in proteins with cleavage sites between amino acids 25 and 26 with SignalP software tool available at the website <http://www.cbs.dtu.dk/services/SignalP>. The other hydrophobic region is predicted to be a transmembrane domain with the TMHMM2.0 program available at the website <http://www.cbs.dtu.dk/services/TMHMM/TMHMM2.0b.guide.html>. Therefore, the NY-SAR-35 gene encodes a signal peptide and a transmembrane domain (Figure 2).

Please amend the paragraph that begins on page 85, line 13, as follows:

The cDNA sequences encoding the 113 sarcoma antigens were compared with sequences deposited in the cancer immunome or SEREX database (<http://www2.licr.org/CancerImmunomeDB>, formerly www.licr.org/SEREX.html). These comparisons are in addition to the comparisons presented above in Example 1. In a preliminary analysis, it was found that 39 of the 113 sarcoma antigens defined in this study (34%) were also identified through SEREX analysis of other tumor types (Table 8). Table 9 below provides a complete list of all 113 antigens along with their respective Unigene cluster information, if any. These results represent the information available after all rounds of immunoscreening. Contrary to the results shown, NY-SAR-39, -57, -61, -63 and -64 after the first round of immunoscreenings had not been found in the SEREX database.

Please amend the paragraph that begins on page 86, line 1, as follows:

AML, acute myelogenous leukemia; BC, breast cancer; CC, colon cancer; GC, gastric cancer; GL, glioma; HCC, hepatocellular carcinoma; HN, head and neck cancer; LC, lung cancer; MEL, melanoma; OC, ovarian cancer; PC, prostate cancer; PN, pancreatic cancer; RC, renal cancer; SRC, sarcoma; TALL, T cell acute lymphocytic leukemia. *Determined by sequence comparisons with the SEREX database (www2.licr.org/CancerImmunomeDB/).